Biparental immune priming in the pipefish *Syngnathus typhle*

Table S3. Results from ANOSIM analysis of 29 immune genes to test for F0-parental and F1-offspring treatment effects in four-month-old F1-juveniles. Multivariate ANOSIM analysis to assess differences in the gene expression profiles per parental treatment group applying pairwise comparison to relative gene expression data (−ΔCt-values) based on a Bray-Curtis distance matrix and 999 permutations. Significant p-values are presented in boldface, "ns" indicates no statistically significatn difference. Shown are overall R and significance values over two main treatments (F0-parents and F1-offspring) and their interactions. These are followed by pairwise comparisons between parental control (Control), paternal *Vibrio* (PatV+) and *Tenacibaculum* (PatT+), maternal *Vibrio* (MatV+) and *Tenacibaculum* (MatT+), biparental (Bi-(MatV+PatT+); Bi-(MatT+PatV+)) treatment groups as well as pairwise comparisons between direct offspring treatments *Vibrio* (F1-V+), *Tenacibaculum* (F1-T+), and Naïve (F1-N). ANOSIM analysis was conducted for all target genes (44) and grouped into the following functional gene categories: genes of the innate immune system (13), of the innate and adaptive immune system (5), of the adaptive immune system (8), complement component genes (3).

ANOSIM	Immune genes [29]	Innate [13]	Innate & Adaptive [5]	Adaptive [8]	Complement [3]
F0-parents (7 treatments)					
Overall R	0.156	0.076	0.074	0.179	0.132
Overall significance values	**0.001**	**0.010**	**0.008**	**0.001**	**0.001**
Bi-(MatV+PatT+) x Bi-(MatT+PatV+)	**0.003**	**0.002**	ns	**0.001**	ns
Bi-(MatV+PatT+) x MatV+	**0.006**	**0.028**	ns	**0.004**	ns
Bi-(MatV+PatT+) x MatT+	**0.008**	ns	**0.011**	ns	ns
Bi-(MatV+PatT+) x PatV+	**0.027**	ns	ns	**0.003**	ns
Bi-(MatV+PatT+) x PatT+	ns	ns	ns	ns	ns
Bi-(MatV+PatT+) x Control	**0.029**	**0.015**	ns	**0.013**	**0.004**
Bi-(MatT+PatV+) x MatV+	**0.011**	**0.002**	0.076	**0.015**	ns
Bi-(MatT+PatV+) x MatT+	**0.002**	**0.001**	**0.005**	**0.002**	ns
Bi-(MatT+PatV+) x PatV+	ns	ns	ns	ns	ns
Bi-(MatT+PatV+) x PatT+	**0.003**	**0.014**	ns	**0.007**	ns
Bi-(MatT+PatV+) x Control	**0.001**	**0.008**	ns	**0.001**	**0.006**
MatV+ x MatT+	**0.001**	**0.006**	**0.009**	**0.001**	**0.047**
MatV+ x PatV+	ns	ns	ns	**0.015**	ns
MatV+ x PatT+	**0.044**	**0.037**	ns	ns	ns
MatV+ x Control	**0.001**	**0.004**	**0.009**	**0.001**	**0.001**
MatT+ x PatV+	**0.001**	**0.031**	**0.002**	**0.036**	**0.003**
MatT+ x PatT+	**0.006**	ns	**0.004**	**0.014**	ns
MatT+ x Control	**0.010**	ns	**0.008**	**0.044**	0.067
PatV+ x PatT+	**0.028**	ns	ns	**0.032**	**0.048**
PatV+ x Control	**0.001**	0.064	ns	**0.001**	**0.001**
PatT+ x Control	ns	ns	ns	**0.012**	0.024
F1-offspring (3 treatments)					
ANOSIM-overall R	**0.056**	**0.076**	**0.013**	**0.027**	ns
Overall significance values	**0.001**	**0.001**	**0.048**	**0.006**	ns
F1-V+ x F1-T+	ns	ns	ns	**0.030**	ns
F1-V+ x F1-N	**0.001**	**0.001**	**0.042**	**0.027**	ns
F1-T+ x F1-N	**0.001**	**0.001**	**0.039**	**0.008**	ns
F0-parents x F1-offspring (interaction)	ns	ns	ns	ns	ns